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AN EXAMINATION OF THE TREPONEMAL IMMOBILIZATION TEST IN THE INVESTIGATION OF POSITIVE SEROLOGICAL TESTS FOR SYPHILIS IN PREGNANCY*†

AN INVESTIGATION CARRIED OUT BY
THE BRITISH CO-OPERATIVE CLINICAL GROUP

The occurrence of a positive serological test for syphilis (STS) on routine antenatal testing raises a difficult diagnostic problem when, as is often the case, clinical investigation fails to explain the serological findings and the possibility of the positive reactions being non-specific has to be considered. The Treponemal Immobilization Test (TPI), which detects a specific anti-treponemal antibody, is widely used as a verification test to check the specificity of positive STS and the present investigation was undertaken to try to correlate the results of the TPI test on sera from pregnant women whose routine antenatal tests had been found positive with the clinical information available, and so to attempt to assess its reliability in this type of case.

The incidence of positive antenatal STS in Great Britain is low, the figure for 1955 being 0·21 to 0·43 per cent. (Chief Medical Officer, 1957), so that the clinical material in individual clinics is limited. This study was therefore undertaken by the British Co-operative Clinical Group, which is sponsored by the Medical Society for the Study of Venereal Diseases, and data were collected from 58 clinics. The Directors of these clinics were asked to complete the questionnaire (Figure, opposite) for all pregnant women who had been referred to them between Jan. 1, 1955, and June 30, 1956, because antenatal serum tests had been found positive, irrespective of whether TPI tests had been performed or not. As this test has not been generally available, it was felt that the patients on whom it was carried out might not be representative, and a control series of patients was therefore desirable.

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† The Report was prepared on behalf of the Group by Dr. A. E. Wilkinson, V.D. Reference Laboratory (M.R.C.).

Clinical Material

514 completed questionnaires were returned. Eleven of these were rejected, four because the patients were not sero-positive, three because of lack of details, one because the same patient attended two clinics, two because the data referred to a second pregnancy during the period under review and details of these two patients' first pregnancies had been included already, and one because the country of origin of the patient was not recorded. Thus, data regarding 503 case reports were available for analysis. The patients included 423 of British or European nationality and eighty coloured people, mainly from the West Indies or West Africa.

Serological Tests

TPI tests were performed on sera from 177 of the 423 white patients and from 44 of the eighty coloured patients. These tests were performed at the Royal Free Hospital on the sera of 25 patients referred to the V.D. Clinic there, and the remainder were done at the V.D. Reference Laboratory (Medical Research Council). The technique used was the same in both laboratories. In addition, a Wassermann reaction using crude heart extract antigen, a standard Kahn test, and Price's precipitation reaction were performed on the specimens of sera which were used for the TPI tests.

The two racial groups of white and coloured patients were each divided into two sub-groups:

(*a*) Those who had had a TPI test performed,
(*b*) Those for whom this was not done.

Analysis with regard to age, previous pregnancies, previous miscarriages or stillbirths, and the incidence of complications of pregnancy showed no significant differences between the sub-groups (*a*) and (*b*) in

BRITISH COOPERATIVE CLINICAL GROUP

Clinical and serological investigation of women found to have positive STS on routine antenatal testing, with special reference to the treponemal immobilization test (TPI).

Clinic:..... Patient's Identification No.:.....

Age:..... Race:..... Country of Origin:.....

A. History

(1) History of Past Syphilis: .....

past yaws: .....

(2) Previous Pregnancies: Children: ..... Number: ..... Ages: .....

Miscarriages Number: ..... Dates: .....
months gestation

(3) Had STS been performed in Previous Pregnancies?.....

B. Serological Investigations During Present Pregnancy

	At Local Laboratory	At Reference Laboratory *
Date. Complement Fixation:	.....	.....
Flocculation:	.....	.....
TPI	.....	.....
Other (stipulate)	.....	.....

Duration of pregnancy when STS found positive .....

Were there any complications of pregnancy? .....

C. Clinical Investigation of Patient

(1) Was there any clinical evidence suggestive of:

a. Congenital syphilis? .....

b. Acquired syphilis? .....

c. History of other venereal disease? .....

(*a.b.c.* If "yes", give details)

(2) Has the patient received penicillin in the past?.....

(3) Could the duration of infection be estimated? (stipulate).....

(4) Was any condition noted which might cause non-specific STS reactions? (stipulate).....

D. Family Investigation

	Results of Clinical Examination	STS results
Husband:	.....	.....
Patient's mother:	.....	.....
Patient's father:	.....	.....
Patient's siblings:	.....	.....
Patient's children:	.....	.....

E. Was anti-syphilitic treatment given to the patient?

Give details .....

Subsequent STS or TPI results .....

F. Outcome of pregnancy Date of birth:.....

Did child show clinical evidence of congenital syphilis?.....

Did child show radiological evidence of congenital syphilis?.....

Results of serum tests on child, and dates.....

G. Diagnosis

Leaving the TPI test out of consideration, what was your *clinical* assessment of the case?

a. Syphilitic

b. Non-specific STS reactions

Remarks:

Date:..... Signed: .....

Clinic:.....

* London Hospital or Royal Free Hospital Laboratories.

white or coloured patients, and it appeared that, as far as these factors were concerned, the patients who had had TPI tests performed could be considered as a fair sample of the total. However, an examination of the white patients with regard to a history of past syphilis showed that the TPI test had been performed on a disproportionate number of patients *without* such a history and in less than the expected number of patients with a history of past syphilis. This difference was significant, but was to be expected in a retrospective study.

Results

The results of the TPI tests in the two racial groups are shown in Table I.

TABLE I
RESULTS OF TPI TESTS IN WHITE AND COLOURED PATIENTS

TPI Result	Race			
	White		Coloured	
	No.	Per cent.	No.	Per cent.
Reactive { Positive ..	125	71	38	88
Reactive { Doubtful ..	5	2·8	0	
Negative	46	26·1	5	12
No Valid Test* ..	1	(Serum infected)	1	(Serum toxic)
Total	177		44	

* These two patients are excluded from the subsequent analysis.

Correlation of the Results of the TPI Test with Other Evidence of Syphilitic Infection

(1) *Patients showing Clinical Evidence or with a History Suggestive of Syphilis.*—These are presented in Table II.

TABLE II
COMPARISON OF TPI RESULTS WITH CLINICAL OBSERVATIONS, BY RACE

Race		White		Coloured	
TPI Result		Reactive	Negative	Reactive	Negative
Clinical Evidence or History Suggestive of	Congenital Syphilis	24	3	1	0
	Acquired Syphilis	7	0	1	1
	Other Venereal Disease	5	0	0	0
	None	93	42	36	4
	Not recorded ..	1	1	0	0
Total		130	46	38	5

Three of the TPI-negative white patients aroused suspicions of congenital syphilis. The mother of one patient was sero-positive and had late latent syphilis, which would help to substantiate the diagnosis. In the second case the physician described the patient's facies as "?? suggestive", but assessed the case as "probably non-specific STS reactions". The third patient was described as "vaguely suggestive" of congenital syphilis. No details of investigations of the family were available, and an antenatal test in a previous pregnancy was known to have been negative. The clinician stated "clinically I might have accepted a diagnosis of congenital syphilis". Thus, in only one of the 45 white patients with negative TPI tests on whom information was available was there any convincing clinical evidence of syphilitic infection. In contrast, 31 out of the 130 TPI-positive patients had signs or histories suggestive of syphilis.

One 30-year-old West African patient, whose TPI test was negative and whose serum gave a positive Kahn test but negative Wassermann reaction and Price's precipitation reaction, was noted to have small irregular pupils which reacted sluggishly to light and accommodation, arousing suspicion of syphilis. No treatment was given; she was delivered of a macerated foetus in which the pathologist found no evidence of syphilis.

(2) *Results of Investigations of the White Patients' Families.*—The results of clinical and serological investigations are shown in Tables III and IV. The information about the coloured patients' families is so limited that it has been omitted.

TABLE III
RESULTS OF CLINICAL EXAMINATION OF PATIENTS' FAMILIES

TPI Result		Reactive (130 cases)			Negative (46 cases)		
Evidence		Yes	No	No Information	Yes	No	No Information
Relationship	Husband	9	63	58	1	21	24
	Mother	4	12	114	1	3	42
	Father ..	3	4	123	0	1	45
	Sibling ..	2	4	124	0	1	45
	Child ..	2	22	106	0	7	39

TABLE IV
RESULTS OF SEROLOGICAL INVESTIGATION OF PATIENTS' FAMILIES

TPI Result ..		Reactive (130 cases)			Negative (46 cases)		
STS Result* ..		Positive	Negative	No Information	Positive	Negative	No Information
Relationship	Husband	8	77	45	1	24	21
	Mother	11	12	107	1	3	42
	Father	2	7	121	0	0	46
	Sibling ..	3	8	119	0	2	44
	Child ..	6	24	100	0	10	36

* In almost all instances, the STS results are those obtained at local laboratories.

In the group of 46 TPI-negative patients, two members of the families showed evidence of syphilis. One patient, a suspected congenital syphilitic whose mother was sero-positive, has already been mentioned. The second was a woman aged 42 whose husband was sero-positive and who had been diagnosed as having syphilis. She showed no clinical evidence of syphilis and had never received penicillin. Negative TPI results were obtained on two separate specimens of serum and the STS were consistently positive at a low titre (Price's precipitation reaction positive with neat serum). She had six living children, aged 5 to 16 years, all of whom were clinically normal and sero-negative. One other child had died in infancy and there had been one stillbirth and one miscarriage. No antenatal tests had been performed in previous pregnancies. Two months after treatment with penicillin and bismuth the Wassermann reaction was doubtful and the Kahn test negative. Subsequent STS and a cerebrospinal fluid examination were negative. She was delivered of a child which showed no clinical or serological evidence of syphilis. This patient was assessed clinically as having latent syphilis.

One or more members of the families of the TPI-reactive patients showed clinical evidence or had a history of syphilis in fourteen instances or were found to be sero-positive in 23 cases.

(3) *Outcome of Pregnancy*.—Anti-syphilitic treatment was known to have been given during pregnancy to 122 of the 130 TPI-reactive patients and after delivery in three instances. Only twelve of the TPI-negative group patients were treated.

In Table V the outcome of pregnancy is shown with regard to the presence or absence of clinical or serological evidence of syphilis in the children born to the TPI-reactive and TPI-negative mothers.

Unfortunately, information about the children born to the TPI-negative mothers who were not treated is lacking in more than half the cases, presumably because the patient's attendance at the V.D. clinic was not considered necessary after it had been decided that her positive STS reactions were non-specific in nature.

In the cases of six of the 130 TPI-reactive mothers, the pregnancy resulted in a dead child: in two cases there was an associated complication of pregnancy (eclampsia, accidental haemorrhage), in two the child showed no evidence of syphilis, and in the remaining two syphilis may perhaps have contributed to the foetal death. One woman who had not been treated gave birth to a stillborn macerated foetus at the 34th week; in the other case, the foetus was dead *in utero* when the mother was first seen, having been treated for primary syphilis 2 months previously. One of the 46 TPI-negative mothers who had not been treated gave birth to a macerated foetus in the fifth month of pregnancy. It showed no clinical evidence of syphilis.

TABLE V

COMPARISON OF THE CLINICAL, RADIOLOGICAL AND SEROLOGICAL FINDINGS IN THE CHILDREN

Children		Mothers		
		TPI-Reactive (130)	TPI-Negative	
			Treated	Not Treated
Status of Child	Living	111	11	15
	Dead	6	0	1
	No record	11	1	15
	Not yet born	2	0	3
Clinical Evidence of Syphilis	Yes	0	0	0
	No	108	9	16
	No record	22	3	18
Radiological Evidence of Syphilis	Yes	1	0	0
	No	26	2	4
	No record	103	10	30
Serological Evidence of Syphilis	Yes	1	1	0
	No	94	9	11
	No record	35	2	23

None of the children who were known to have been born alive showed clinical evidence of congenital syphilis. Few radiological investigations were carried out, but one child showed *x*-ray signs of periostitis, though it was sero-negative when tested 2 months after birth. The mother had been treated late in pregnancy.

Two children had positive serological tests. One was found to have a negative Wassermann reaction and doubtful Kahn test 5 days after birth, its mother was a latent congenital syphilitic whose TPI test was positive and who had been treated during her pregnancy. The second child showed no clinical signs of congenital infection at birth, but was stated to be sero-positive some 14 weeks later; no further tests were recorded. The mother, who was TPI-negative and showed no clinical evidence or family history suggesting syphilis, was given 4·8 mega units P.A.M. during her pregnancy and was sero-negative 4 months after delivery. Five other children were sero-positive at or shortly after birth, but subsequent STS reactions were negative, suggesting that the initial positive reactions were due to passive transfer of maternal antibody. It is of interest that one of the

mothers of these children was TPI-negative, which suggests that the reagin responsible for non-specific STS reactions can also pass the placental barrier.

(4) *Correlation of TPI Result with Clinical Assessment.*—Physicians were asked to assess the cases without taking the TPI results into consideration. The opinions expressed are summarized in Table VI. It will be seen that, in general, the TPI results agree fairly well with the clinical opinions, although the divergencies are greater in the TPI-negative group, as might be expected.

TABLE VI
COMPARISON OF TPI RESULT WITH CLINICAL ASSESSMENT

Opinion	TPI Result	
	Reactive	Negative
Syphilitic	112	6
Non-specific STS Reactions	6	34
Doubtful or "Do not know" ..	12	6

Associated Factors in the Two Groups of Patients

Age.—The average age of the TPI-negative group of patients (27·6 yrs) was slightly lower than that of those found TPI-reactive (30·1 yrs); 50 per cent. of the former group were aged between 21 and 28, compared with 33 per cent. of the latter. The difference in composition of the age groups in the two classes of patients was not statistically significant.

Parity.—The two groups contained approximately equal proportions of primiparae, but the TPI-negative patients included 31 per cent. who had had one previous pregnancy, compared with 16·9 per cent. in the TPI-reactive group. The differences in composition of the two groups of patients regarding parity was not significant.

Duration of Pregnancy when the STS were found Positive.—No differences were noted between the two groups.

Incidence of Complications of Pregnancy.—These were reported in nine of the 130 TPI-reactive patients and in four of the 46 TPI-negative group.

Conditions which might possibly cause Non-specific STS Reactions.—These were recorded in six of the TPI-negative patients:

(*i*) A disease (? rubella) 2 years previously associated with stiffness of fingers and wrists and effusions into the knees
(*ii*) Mild iron-deficiency anaemia
(*iii*) Pregnancy toxaemia
(*iv*) Pneumonia
(*v*) Recent jaundice
(*vi*) Disseminated sclerosis

and in three of the TPI-reactive patients:

(*i*) Severe anaemia
(*ii*) Pulmonary tuberculosis
(*iii*) An obscure interlobal lung lesion of undetermined aetiology.

Previous Treatment with Penicillin.—Five of the TPI-negative mothers were known to have had penicillin in the past, while thirty were stated not to have had the drug, no information being available about the remainder. Of the TPI-reactive mothers, 28 had had penicillin previously, three of them for syphilis, and 77 had never been given the drug before. It seems unlikely, therefore, that the negative TPI-tests were due to unwitting treatment of occult syphilitics with penicillin for conditions other than syphilis.

Pattern of the STS Reactions

Table VII compares the pattern of results given by the three STS in the reactive and negative patients, and Table VIII the strength of the reactions as judged by the Price's precipitation reaction titre.

TABLE VII
COMPARISON OF STS PATTERNS IN THE TPI-REACTIVE AND TPI-NEGATIVE GROUPS

Test		STS Pattern								Total
Wassermann Reaction		+	0	+	+	0	0	0	+	
Kahn Test ..		+	0	0	+	+	0	+	0	
Price's Precipitation Reaction ..		+	0	0	0	0	+	+	+	
TPI	Reactive	117	2	2	0	2	0	1	5	129*
	Negative	17	11	6	3	2	3	2	1	45*

* One serum in group omitted as Price's precipitation reaction not done.

TABLE VIII
COMPARISON OF PPR TITRES IN TPI-REACTIVE AND TPI-NEGATIVE GROUPS

Price's Precipitation Reaction Titre	TPI Result	
	Reactive	Negative
0 (negative)	6	23
Neat serum	29	9
1 in 2	26	6
1 in 4	17	2
1 in 8	21	2
1 in 16 or more	30	3
Total	129*	45*

* No Price's precipitation reaction result available in one serum from each group.

These figures show that there was far closer agreement between the individual STS in the TPI-reactive group of sera, in which the incidence of discrepant STS reactions was 7·8 per cent. as against 37·7 per cent. in sera from TPI-negative patients. It should be noted, however, that in this last group, seventeen sera were positive in all three STS.

These results show that the majority of TPI-negative sera giving positive Price's precipitation reaction results were only of low titre, but that there were a small number of strongly reactive sera, so that the height of the titre is no absolute criterion of specificity, as judged by the TPI result.

Discussion

The finding of a positive serological test for syphilis on routine antenatal testing is frequently unsupported by any other evidence of infection. In the group of 130 TPI-reactive white patients, there was clinical evidence suggestive of syphilis or information from examination of the patients' families supporting the diagnosis in only 54 cases. In the remainder, the diagnosis rested on the serological results alone and the findings of a positive TPI test afforded useful corroborative evidence. In marked contrast, only two of the 46 women whose TPI tests had been found negative showed any convincing evidence of syphilis. One of these was a case of congenital infection, and previous experience has shown that the TPI test may occasionally be negative in this type of case. Although information about the outcome of the pregnancy was unfortunately lacking in about half the TPI-negative patients who were left untreated, the infants who were examined showed no clinical evidence of infection, nor was this found in the one miscarriage which occurred in this group of patients. Thus what clinical findings were available were in agreement with the TPI result and suggested that the positive STS results in this group of mothers were probably non-specific.

Estimates of the frequency with which non-specific STS results are found in pregnancy have varied very widely. The earlier work has been exhaustively reviewed by Penttinen (1947). This author examined over 18,000 pregnancy sera in Helsinki from 1935–45 and estimated that 18·8 per cent. of the positive STS reactions he found were non-specific. Pigeaud, Sohier, Thivolet, Richard, and Rolland (1954) noted that the TPI test was negative in thirteen of a small group of sixteen cases, where the antenatal STS had given discordant results. Wheeler, Van Goor, and Curtis (1954) reported that 29 out of 39 pregnant women whose antenatal STS was positive were found to have negative TPI tests. Boak, Carpenter, Miller, Drusch, Chapman, and Heidbreder (1955) carried out TPI tests on 400 sera from women who had been found sero-positive in pregnancy and found that 73 per cent. were TPI-negative. Lighter (1957) found twelve biological false positive reactors in a group of 28 pregnant women, most of whom were Negroes, and did not consider that pregnancy itself caused biological false positive reaction. Wilkinson and Sequeira (1955) found that 27·5 per cent. of 244 STS-positive antenatal sera gave negative TPI reactions, suggesting that the STS results might be non-specific. This last figure is in close agreement with the results of the present series, in which 23·3 per cent. of negative TPI reactions were found in the combined white and coloured patients. It must be stressed, however, that in both the last series mentioned, the incidence may not reflect the true position as the patients tested were probably selected to an unknown degree. Despite this caveat, it seems clear that an appreciable proportion of positive STS reactions found on routine antenatal testing may be non-specific. With the diminishing incidence of syphilis it seems possible that the proportion of non-specific reactions found may increase in the future.

The possible association of persistent non-specific STS reactions with the so-called collagen group of disorders has been described by Moore and Mohr (1952) and Moore and Lutz (1955). Miller, Brodey, and Hill (1957) studied 555 patients whose sera gave non-specific STS reactions and showed that 71 per cent. of these were in females, the onset being most frequent in the 20 to 30-year age group. 15 per cent. of the whole group were found to have diseases possibly associated with the lupus diathesis or systemic lupus. The present study has shown that many of the non-specific reactions in pregnancy occur in young women; such reactions should not be disregarded; if persistent, they should be investigated to try to find what has provoked them.

The ultimate responsibility for the decision that a positive serological test for syphilis is non-specific rests on the physician and not on the serologist. The diagnosis is one by exclusion and can only be made with any degree of confidence after a thorough investigation of the patient and her family. The TPI test can play an important part in this, but it has its limitations which must be clearly recognized, and it should not be used as a short cut and allowed to usurp the functions of the physician.

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